Rheonix STI TriPlex™ Assay

℞ ONLY

For *in vitro* diagnostic use
Catalog Number KCSTI3-24

For use only with the Rheonix Encompass MDx® Workstation

Package Insert

Rheonix, Inc.
10 Brown Road
Suite 103
Ithaca, NY 14850
Customer Service:
Phone Number: 1-844-RHEONIX (1-844-743-6649)
Email: techsupport@rheonix.com

M23109 Rev BX1

Table of Contents

Rheonix STI TriPlex™ Assay 0

Package Insert 0

Product Name 2

Intended Use 2

Summary and Explanation of the Test 2

Principles of the Procedure 4

Materials Provided/Assay Kit Components 4

- STI TriPlex™ CARD Pack 5
- STI TriPlex™ Reagent Pack (Pack A) 5
- STI TriPlex™ PCR Mix (Pack B) 6
- Materials Required/Recommended, but not Provided 6
- Warnings and Precautions 7

Specimen Collection, Transport and Storage 8

- Sample Collection 8
- Sample Transport and Storage 8

Instructions for Use 8

Quality Controls 11

Results/Test Interpretation 11

Meaning of Error or Indeterminate Codes 14

Limitations 15

Expected Values 16

Performance Characteristics 16

Positive Predictive Values/Negative Predictive Values 21

Repeat and Unresolved Rates 21

ANALYTICAL PERFORMANCE 22

- Analytical Sensitivity 22
- Inclusivity 22
- Analytical Specificity/Cross Reactivity 24
- Interfering Substances 26
- Mixed Infection/Competitive Interference 27
- Precision and Reproducibility 27

Carry Over/Cross-contamination 30
Labeling Symbology 32
Intellectual Property 33

Product Name

Rheonix® STI TriPlex™ Assay

Intended Use

For *in-vitro* Diagnostic Use.
The Rheonix STI TriPlex™ Assay, as performed on the Rheonix Encompass MDx® Workstation, is an automated DNA extraction and multiplex PCR amplification test system intended for the direct, qualitative detection of DNA from *Chlamydia trachomatis* (CT), and/or *Neisseria gonorrhoeae* (NG), and/or *Trichomonas vaginalis* (TV) in male urine specimens collected with the Rheonix Urine Specimen Collection Kit. The test is indicated to aid in the diagnosis of chlamydial urogenital disease, gonococcal urogenital disease and trichomoniasis in asymptomatic or symptomatic male individuals.

Summary and Explanation of the Test

The information below is derived from the "Sexually Transmitted Infections Treatment Guidelines, 2021.", released by the Centers for Disease Controls and Prevention.

https://www.cdc.gov/std/treatment-guidelines/STI-Guidelines-2021.pdf

Chlamydia trachomatis (CT)

Chlamydia is a common sexually transmitted disease (STD) caused by infection with *Chlamydia trachomatis*. *Chlamydia trachomatis* are gram-negative, obligate intracellular bacteria that form characteristic intracellular inclusions which can be observed in cell culture by fluorescence microscopy. It can cause cervicitis in women and urethritis and proctitis in both men and women. Chlamydial infections in women can lead to serious consequences including pelvic inflammatory disease (PID), tubal factor infertility, ectopic pregnancy, and chronic pelvic pain. Lymphogranuloma venereum (LGV), another type of STD caused by different serovars of the same bacterium, occurs commonly in the developing world, and has more recently emerged as a cause of outbreaks of proctitis among men who have sex with men (MSM) worldwide.

According to the latest STD Surveillance Report published in April 2021 from the US Centers for Disease Control and Prevention (CDC), chlamydia infection is the most common notifiable disease in the United States with over 1.8 million cases reported to the CDC in 2019 and represents the largest of all STDs reported to CDC. During 2018–2019, rates of reported chlamydia increased among both males and females, in all regions of the United States, and among all racial/Hispanic ethnicity groups. Although rates of reported cases among men are generally lower than rates among women, rates among men increased 32.1% during 2015–2019.

Neisseria gonorrhoeae (NG)

The information below is derived from the "Sexually transmitted Infections Treatment Guidelines, 2021", released by the Centers for Disease Controls and Prevention.

https://www.cdc.gov/std/treatment-guidelines/STI-Guidelines-2021.pdf

Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance 2019. Atlanta: US Department of Health and Human Services; 2021.

Neisseria gonorrhoeae are gram-negative, oxidase-positive diplococci that can be observed in Gram-stained smears of urethral discharge, usually within neutrophils. Infection by *Neisseria gonorrhoeae* is caused by sexual contact with the penis, vagina, mouth or anus of an infected partner. Ejaculation does not have to occur for gonorrhea to be transmitted or acquired. Moreover, gonorrhea can also be spread perinatally from mother to baby during childbirth. In some instances, males with gonorrhea may not exhibit any symptoms. However, symptomatic males may present with symptoms including a burning sensation when urinating, a white, yellow, or green discharge from the penis or painful or swollen testicles (which is less frequent). Culture of *Neisseria gonorrhoeae* can be difficult because the organism does not survive long outside the host and is highly susceptible to adverse environmental conditions such as lack of humidity and temperature extremes. The CDC estimates that approximately 1,568,000 new *N. gonorrhoeae* infections occur each year.

Trichomonas vaginalis (TV)

The information below is derived from the "Sexually Transmitted Infections Treatment Guidelines, 2021.", released by the Centers for Disease Controls and Prevention.

https://www.cdc.gov/std/treatment-guidelines/STI-Guidelines-2021.pdf

Trichomonas vaginalis is a parasitic protozoan that can be transmitted from an infected individual to a sexual partner. Infected men may notice itching or irritation within the penis, burning after urination or ejaculation or a discharge from the penis. *T. vaginalis* can cause urethritis among heterosexual men; however, the prevalence varies substantially by U.S. geographic region, age, and sexual behavior and within specific populations. Studies among men with and without overt urethritis in developed countries document relatively low rates of *T. vaginalis* in the Netherlands (0.5%), Japan (1.3%), the United States (2.4%), and the United Kingdom (3.6%). Studies in other countries have documented higher rates, such as in Croatia (8.2%) and Zimbabwe (8.4%), particularly among symptomatic patients. Although it is the most common curable sexually transmitted infection, only about 15-30% of infected individuals develop symptoms which range from mild irritation to severe inflammation. Between 70-85% of patients infected with *T. vaginalis* are asymptomatic. Infections that are not treated could persist from months to years. Considering this, screening is important, since *T. vaginalis* has been associated with reproductive morbidity, higher likelihood of preterm birth (1.4-times higher), premature rupture of membranes, and infants at gestational age who are small.

This device is a Nucleic Acid Amplification Test (NAAT) which represents a more sensitive test method for STI diagnosis compared to the microscopic evaluation of wet mounts and culture methods.

Principles of the Procedure

The Rheonix STI TriPlex™ Assay is an *in vitro* diagnostic test capable of detecting the presence of CT, NG, and/or TV in male urine. The assay uses proprietary Rheonix CARD® cartridge technology that provides a microfluidic network complete with pumps, valves, and reaction chambers for automated assay performance. Each CARD cartridge provides assay chambers for four separate clinical specimens. The Rheonix Encompass MDx® Workstation can simultaneously process 6 CARD cartridges, for a total of up to 24 specimens per test run. All residual liquids are contained within the device and discarded with the Rheonix STI TriPlex™ Assay consumables, thus optimizing workflow and minimizing cross contamination.

At the site of collection, the collected clinical specimens are transferred to the urine transport tube (part of the Rheonix Urine Specimen Collection Kit) where cell lysis begins. Once the clinical specimens are lysed by the transport buffer and introduced into the workstation, DNA is extracted, magnetically purified, and delivered to PCR thermocycling chambers. All these are automatically performed on the microfluidic CARD cartridges where each sample lane has two separate PCR chambers into which equal amounts of DNA are metered. One PCR chamber will amplify CT and TV as well as the corresponding CT and TV Process Control (PPCs), while the second PCR chamber amplifies NG and NG PPC. To detect NG and TV, the genes coding for ribosomal RNA targets are PCR amplified and to detect CT, cryptic plasmid DNA is PCR amplified. In all cases, the target genes are amplified in the presence of biotin-tagged primers and the resulting amplicons were denatured and flowed over the low-density array of capture probes contained within the CARD cartridge. Following incubation with streptavidin conjugated horseradish peroxidase and substrate, color precipitated spots are detected and analyzed via the onboard image capture system and results provided by the workstation's software as either positive (POS), negative (NEG), indeterminate (IND), or error (ERR) for each of the three target microorganisms (Figure 1).

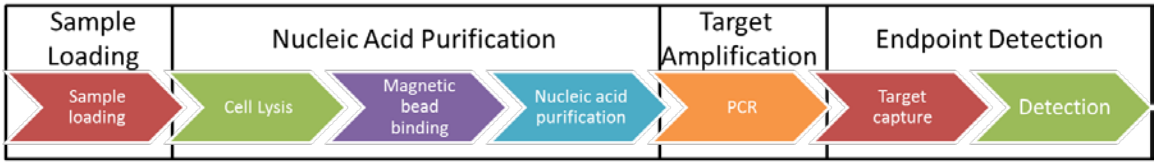


Figure 1. Flowchart for Rheonix STI-Triplex assay. All steps, performed automatically on the Encompass MDx workstation, are shown.

Materials Provided/Assay Kit Components

The following packaged consumables are supplied in the Rheonix STI TriPlex™ Assay Kit (Catalog No. KCSTI3-24) to run up to 24 specimens in a batch mode. Store all kit components at room temperature (15 °C to 30 °C).

- 2 STI TriPlex™ CARD Packs of 3 cartridges each (Part No: M16034*)
- 1 STI TriPlex™ Reagent Pack (Pack A) (Part No: M21745*)
- 1 STI TriPlex™ PCR Mix (Pack B) (Part No: M16321*)
- 1 Package Insert (Part No: M23109*)

**Components not sold individually*

STI TriPlex™ CARD Pack

Each STI TriPlex™ CARD Pack consists of three CARD cartridges in a Tyvek®-sealed plastic tray (Figure 2). Each assay kit contains two CARD Packs.

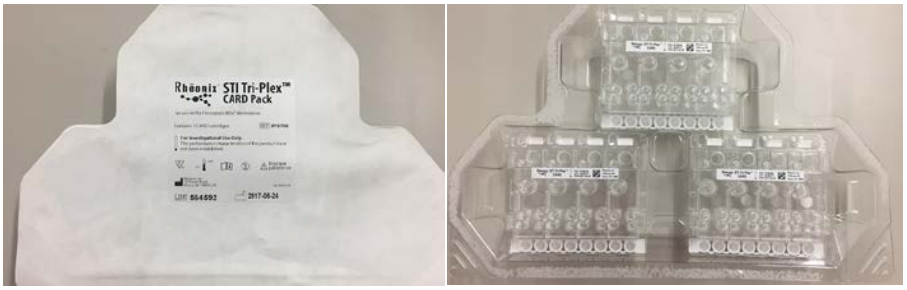


Figure 2. STI TriPlex™ CARD Pack

STI TriPlex™ Reagent Pack (Pack A)

The STI TriPlex™ Reagent Pack (Pack A) contains seven sealed reagent tube strips and two 200µL pipet tips (Figure 3). The STI TriPlex™ Reagent Pack is sealed with a Tyvek cover. See Table I for a detailed description of the contents of the Reagent Pack.

Figure 3. STI TriPlex™ Reagent Pack (Pack A)

Table I. Contents of STI TriPlex™ Reagent Pack (Pack A)

Reagent	Amount	Unit of Measure	Quantity
Deionized Water	9.2	mL	1
Elution Buffer	2.5	mL	1
96% Glycerol	6.5	mL	1
HRP	600	µL	1
Isopropanol	7.2	mL	1
Lysis Buffer	6.8	mL	1
Magnetic Beads	421	µL	1

Mineral Oil	1000	µL	2
Proteinase K	300	µL	1
PCR and Process Control (PPC)	310	µL	1
Sodium Hydroxide	3.5	mL	1
SS Buffer	8.9	mL	5
TMB	4.6	mL	1
Wash 1	7.5	mL	1
Wash 2	9.3	mL	1
200 µL Pipette Tip	N/A	N/A	2

The Reagent Pack also contains an empty strip slot running perpendicular to the other seven tube strip slots for securing the STI TriPlex™ PCR Mix strip (Strip B) into the Reagent Pack via a hinged latch.

Each Reagent Pack can perform testing for up to 24 samples.

Store the STI TriPlex™ Reagent Pack in its box in an upright position.

STI TriPlex™ PCR Mix (Pack B)

Each STI TriPlex™ PCR Mix (Pack B) is supplied in a foil-sealed pouch. The PCR Mix tube strip contains both PCR Mix 1 (used to amplify CT and TV DNA and controls) and PCR Mix 2 (used to amplify NG DNA and control) in the form of lyophilized beads (Figure 4). PCR Mix 1 and PCR Mix 2 both contain DNA polymerase, nucleotides and CT/NG/TV specific molecular primers along with control-specific primers.

Figure 4. STI TriPlex™ PCR Mix (Pack B)

The plastic tabs at either end of the STI TriPlex™ PCR Mix strip are keyed to ensure correct insertion and orientation into Reagent Pack A.

Each PCR Mix strip is sufficient to analyze up to 24 samples.

Materials Required/Recommended, but not Provided

The following items are not provided, but required/recommended:

- Rheonix Encompass MDx Workstation (Rheonix Catalog Number RNXMDX) (required)
- Rheonix Urine Specimen Collection Kit (Rheonix Catalog Number CCUR-50) (required)
- External Quality Controls
- Axygen 1000 µL tips (Axygen/TTF-1000-C-HTR-S; VWR/89040-092)

Warnings and Precautions

- The Rheonix STI TriPlex® Assay is for *in vitro* Diagnostic Use and must be performed using the Rheonix Encompass MDx™ Workstation within the appropriate operating conditions.

Environmental Conditions - Operation	
Temperature	18° C-30° C / 64° F-86° F
Humidity	20 to 85% non-condensing
Altitude	1600 m maximum

- The Rheonix STI TriPlex® Assay is for use only with Rheonix Urine Specimen Collection Kit (Rheonix Catalog Number CCUR-50) for Male Urine.

- The Rheonix STI TriPlex™ Reagent Pack contains guanidine hydrochloride, a chaotropic agent that is widely used for purification of proteins and nucleic acids. In case of skin contact with these reagents, remove contaminated clothing and wash the affected area with soap and water. In case of eye contact, flush eyes thoroughly with water for at least 15 minutes. Consult a physician.
- Do not use expired kits.
- Do not use the kit if the seal to the outer box is broken upon arrival.
- Do not use any kit components that display damage or broken seals.
- Do not mix reagents from one kit with another kit.
- The laboratory should perform routine environmental monitoring to minimize the risk of cross contamination.
- Although the kit contains an internal PCR and Process Control, the use of well-characterized or commercially available controls may be used at the discretion of the testing laboratory.
- All specimens should be handled by operators as if they are infectious and in accordance with safe laboratory procedures.
- Operators should wear protective clothing, disposable gloves and safety glasses. The Rheonix Encompass MDx Workstation's User Interface provides instructions on when to change disposable gloves during the testing procedure. Strict adherence to the instructions is required to protect the operator and reduce possible erroneous results.
- Thoroughly wash hands after performing the tests
- There is no need to pipette any reagents.
- Do not eat, drink, smoke, chew in areas where specimens or kits are being used.
- Dispose of all consumable test components and waste in accordance with local, state and/or federal regulations.

- The lab performing the testing must have a spill kit on site, to safely clean up spills of potentially hazardous materials
- Safety Data Sheets (SDS) are available at Customer Service at (Phone number: 1-844-RHEONIX (1-844-743-6649)

Specimen Collection, Transport and Storage

The specimens tested with the Rheonix STI TriPlex™ Assay must be collected using the Rheonix Urine Specimen Collection Kit (Rheonix Catalog Number CCSW-50).

Sample Collection

Please refer to the appropriate specimen collection kit package insert for collection instructions.

Sample Transport and Storage

1. Urine Specimens
   Using the disposable pipette, transfer 3 mL from the urine specimen cup into the transport buffer immediately after collection and store at 2°C to 30°C. Although it is recommended that testing be completed within seven days after collection, studies indicate that the specimens, when properly stored, provide valid results up to 120 days after collection, when stored at 2°C to 8°C or up to 75 days when stored at 30°C.
   - In the event a sample needs to be reanalyzed, the test should be repeated within 120 days of the initial test when stored at 2°C to 8°C, or within 75 days when stored at 30°C.

Instructions for Use

- **Caution:** In order to reduce the chance for contamination, do not open assay consumables (CARD Pack, Reagent Pack A and Reagent Pack B) until prompted by the User Interface (UI).
- The assay must be run on the Rheonix Encompass MDx® Workstation.
- Assay run time is approximately 6 hours.
- Gloves and other appropriate personal protective equipment should be worn at all times during preparation and running of the assay. The touch screen can be manipulated while wearing gloves.

The following instructions provide the primary steps for conducting the STI TriPlex™ Assay on the Encompass MDx® Workstation. For detailed information on Encompass MDx Workstation operation, refer to *Encompass MDx® Workstation Operator Manual.*

Assay Steps

1. Ensure the Encompass MDx® Workstation has been cleaned according to the instructions provided in the Encompass MDx® Workstation Operator Manual.
2. Start the User Interface Software by powering on the Encompass MDx® Workstation. The power on switch is located in the rear, lower left corner of the workstation.
3. Log in when prompted by the workstation.

4. To begin a new run, select "New Run" from the home screen, then select "Enter."
5. Load up to 24 specimens that were collected as stated in the collection kit package insert and stored between 2°C and 30°C prior to testing into a cleaned Encompass MDx Sample Rack. Consult the Operator Manual for rack cleaning instructions.
6. Ensure that the specimen tubes are inserted into the rack such that the barcodes are centered in the open side of the rack and visible to the operator.
7. Place the sample rack containing the test specimens into the workstation deck as instructed by the UI. Push down gently.
   a) **Do not force the rack into the workstation deck.** If met with resistance upon loading, the sample rack may be in the incorrect orientation.
8. Select the test or tests to perform for the male urine specimen.
   a) The "All CT" and "All NG" boxes are selected by default, while the "All TV" box is unselected by default.
   b) After selecting all appropriate tests, touch "Confirm."
9. After pressing "Confirm," follow the User Interface prompts and load CARD cartridges.

Caution: Before proceeding to load remaining consumables, change your gloves.

10. Remove CARD Packs from the kit using the touch points as shown in Figure 5.

Caution: Do not touch the array covers on the CARD cartridges.

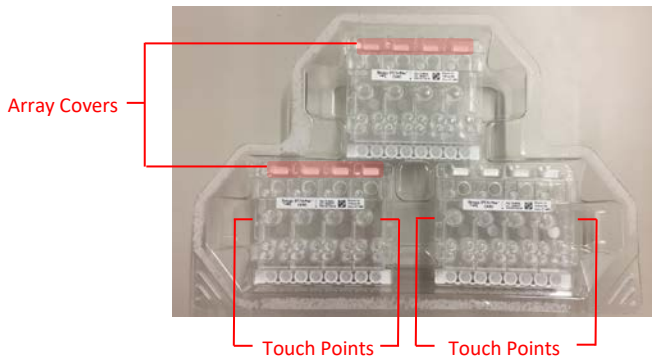


Figure 5. STI TriPlex™ CARD Touch Points and Array Covers

11. Load CARD cartridges with the PCR tube strip facing operator and close clamps.
    a) A test run requires 6 CARD cartridges to be loaded, for a total of up to 24 specimens, per test run.
    b) Partial runs (less than 24 samples) can be performed, however a full assay kit (6 CARDS and an entire reagent pack) must be used. **Partial reagent packs cannot be reused.**
12. **Mix the reagent Pack.** Prior to removing the Tyvek® lid from Reagent Pack A, grasp the pack and use your gloved hands to invert the entire pack three times to mix.
13. Remove the lid from STI TriPlex™ Reagent Pack (Pack A).
    Caution: Do not use if any foil sealant is compromised or the reagent strips are not secured in place upon opening.

Caution: Do not remove reagent strips from the reagent pack or touch the foil sealant covering the reagent strips.

Caution: Do not use Reagent Packs leftover from a previous run.

14. Remove STI TriPlex™ PCR Mix (Pack B) from its foil pouch.
15. Before placing Reagent Pack B into Reagent Pack A, assure that all five lyosphere beads in each of two tubes have not adhered to the foil cap. Bead attachment to the foil cap may prevent them from being resuspended during the assay that could lead to less than optimum concentrations of PCR reagents. If lyosphere beads have attached to the foil cap, gently tap the Pack B to detach them. Figure 6 shows the incorrect location of lyosphere beads and Figure 7 shows the correct location of lyosphere beads.
16. Insert Reagent Pack B into Reagent Pack A by securing with plastic tab on Reagent Pack A.
17. Load the fully assembled Reagent Pack (now consisting of Reagent Pack A and Reagent Pack B) onto the workstation deck.

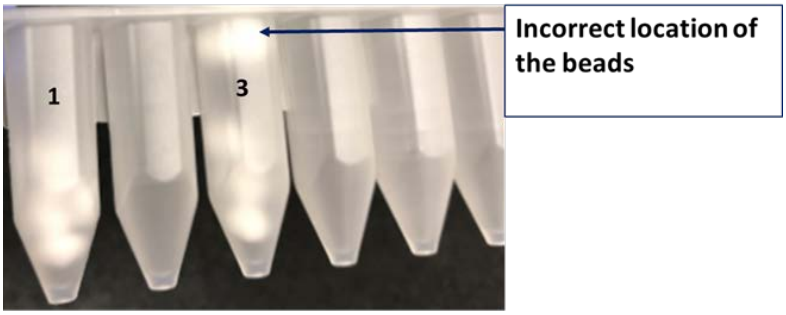


Figure 6. Improper location of lyosphere beads, shown adhering to the upper foil cap.

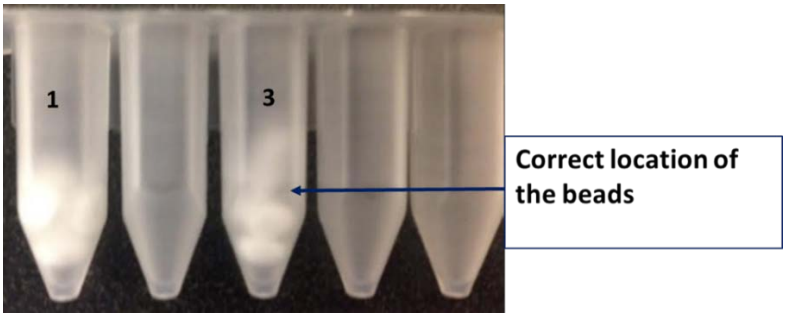


Figure 7. Proper location of lyosphere beads, shown at the bottom of the tubes.

18. Place the two tip tubs onto the workstation deck as indicated by the User Interface. **Caution:** Ensure that at least one of the tip boxes contains a full rack of 96 Axygen tips before continuing.
19. When prompted, manually close the workstation door to initiate the Pre-Run Checks.
20. Touch "Next" on the "Pre-Run Checks Successful" screen and then touch "Start" on the "Run Monitor" screen to start the assay.
21. When the test is complete, the UI displays the "Test Complete" screen:
    a) To review the results in greater detail, touch the "Review" button.
    b) Red squares correspond to positive test results, green squares correspond to negative test results and blue squares correspond to indeterminate results. Dark grey squares indicate that the test was not selected.
22. Test results can either be printed or sent to a USB.

a) **Note**: All data are saved on the Encompass MDx® Workstation unless removed by an administrator.

23. Once finished with the results, unload the deck by removing all samples and used consumables. Dispose of the consumables into properly labeled biohazard trash receptacles; process trash in accordance with all institutional practices and local, state, and federal regulations.

Quality Controls

The Rheonix STI TriPlex™ Assay includes two sets of internal controls. In addition, a laboratory can also run an external negative and/or positive control (not provided by Rheonix).

- Reference Spots (RS) – Three reference spots are included on each microarray. The locations of these spots permit the camera to properly align itself during image analysis. In addition, the Rheonix Encompass MDx Workstation uses information from the image analysis to confirm that all necessary detection reagents performed properly during the run of the assay.

- PCR and Process Control (PPC) – An *Escherichia coli* clone, harboring a chimeric plasmid containing three unique target sequences flanked by primers for each of the target analytes, is added after the clinical specimens are introduced. The PPC is at a concentration low enough to be amplified and detected, yet not compete with the true analyte. The presence or absence of the PPC will be confirmed via detection by specific probe capture on the microarray. The purpose of the PPC is to demonstrate that all steps from extraction to detection, including the PCR amplification, are functioning properly. Additionally, this control detects inhibition of the PCR assay. The Rheonix Encompass MDx Workstation's software requires that the PPC yield a positive result in order for a test specimen to be scored as negative for the target(s) being analyzed. Due to the potential for competition from authentic targets present in the specimen, the PPC result may be positive or negative since high concentrations of target(s) in the clinical specimens may compete with the PPC and yield a negative PPC result. The PPC passes if it meets the acceptance criteria in the software algorithm. Each CARD cartridge sample lane has two separate PCR reactors. One PCR chamber will amplify CT and TV as well as the corresponding CT and TV PPCs, while the second PCR chamber amplifies NG and NG PPC.

- If desired, external positive and negative controls can be run on the Encompass MDx Workstation. The controls can either be well-characterized clinical specimens or purchased from ZeptoMetrix, Inc. (Buffalo, NY). The commercially available positive control (ZeptoMetrix catalog number *abc*) should yield a positive result for all three targets (CT, NG, and TV) while the negative control (ZeptoMetrix catalog number *def)* should yield a negative result for all three targets.

Results/Test Interpretation

The Rheonix Encompass MDx® workstation software automatically evaluates the control and targets according to software algorithms. Based on the algorithms, the Rheonix STI TriPlex™ Assay provides test results for CT, NG, and TV targets as shown in Table II.

Table II. Test Results for the Rheonix STI TriPlex™ Assay.

SPOT DETECTION ON ARRAY							RESULTS REPORTING		
Targets Spots			Process Control Spots			RS			
CT	NG	TV	CT-PPC	NG-PPC	TV-PPC		CT	NG	TV
Yes	Yes	Yes	Yes or No	Yes or No	Yes or No	Yes	+	+	+
Yes	Yes	No	Yes or No	Yes or No	Yes	Yes	+	+	-
Yes	No	No	Yes or No	Yes	Yes	Yes	+	-	-
Yes	No	Yes	Yes or No	Yes	Yes or No	Yes	+	-	+
No	Yes	No	Yes	Yes or No	Yes	Yes	-	+	-
No	Yes	Yes	Yes	Yes or No	Yes or No	Yes	-	+	+
No	No	Yes	Yes	Yes	Yes or No	Yes	-	-	+
No	No	No	Yes	Yes	Yes	Yes	-	-	-
Yes	Yes	No	Yes or No	Yes or No	No	Yes	+	+	?
Yes	No	No	Yes or No	No	No	Yes	+	?	?
Yes	No	Yes	Yes or No	No	Yes or No	Yes	+	?	+
No	Yes	No	No	Yes or No	No	Yes	?	+	?
No	Yes	Yes	No	Yes or No	Yes or No	Yes	?	+	+
No	No	Yes	No	No	Yes or No	Yes	?	?	+
Greyzone intensity	Greyzone intensity	Greyzone intensity	Yes or No	Yes or No	Yes or No	Yes	?	?	?
Yes or No	Yes or No	Yes or No	Yes or No	Yes or No	Yes or No	**No**	!	!	!

Note: *In situations where the target analyte is detected, the Process Control results are ignored (may be either present or absent) because amplification of the respective target (i.e., CT, NG, or TV) can compete with this control.*

CT-Chlamydia trachomatis, NG- Neisseria gonorrhoeae, TV- Trichomonas vaginalis
CT-PPC= process control for Chlamydia trachomatis
NG-PPC= process control for Neisseria gonorrhoeae
TV-PPC= process control for Trichomonas vaginalis
RS= Reference Spot, controls for the integrity of the spot array

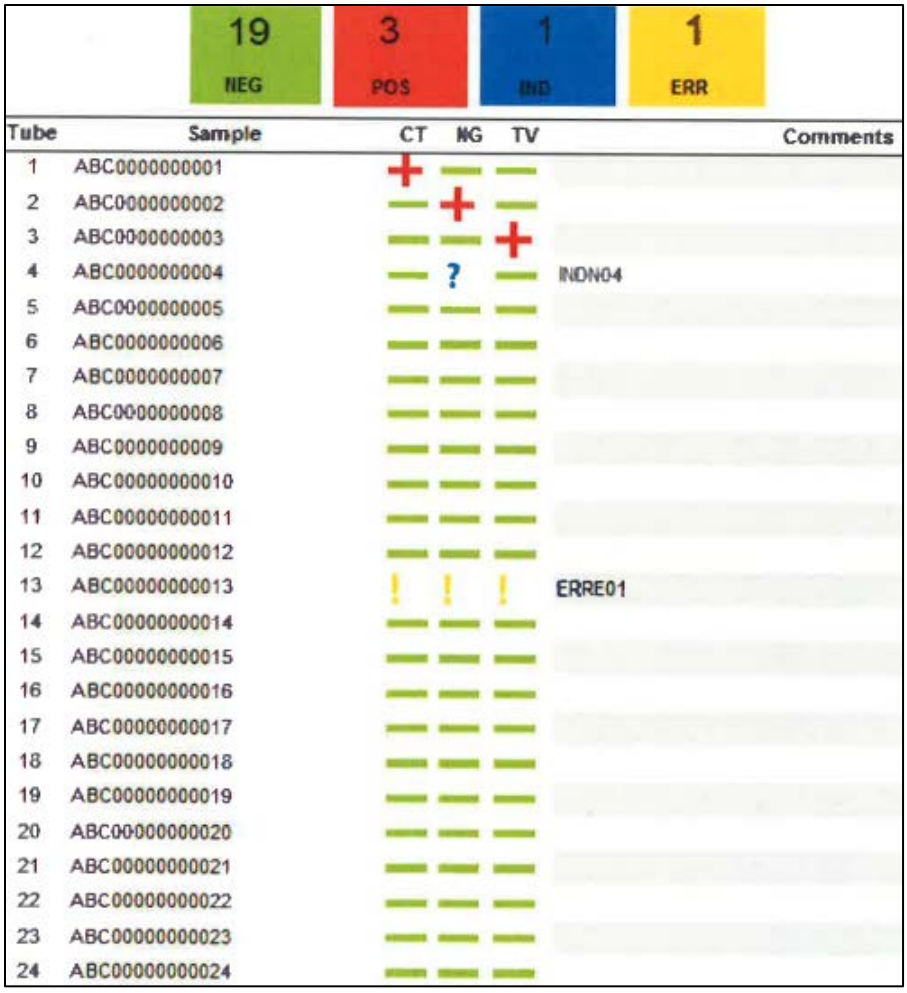
+ = Positive, (Target detected)
- = Negative, (No target detected)
? = Indeterminate, (IND)
! = Error, (ERR)
Greyzone = intensity of hybridization spot is between the upper value of a negative result and the lower value of a positive result

The following table can be used to determine the specific reason for an IND or ERR code:

Code	Explanation

+	**Positive** result (Target detected)
-	**Negative** result (No target detected)
?	**Indeterminate** (IND)
	N02 - Standard deviation (intensity of hybridization spot is not uniform)
	N03 - Lack of one or more Process Controls (needed to confirm a negative result)
	N04 - Grey Zone notification (intensity of hybridization spot is between the upper value of a negative result and lower than the lower value of a positive result
!	**Error**
	E01 - Invalid Reference Spots
	E02 - Failed "bubble check" (presence of bubbles can lead to incorrect result)
	E03 - Failed "spacing" check (can lead to incorrect result)
	E04 - Failed "angle check" (an improper angle between reference spots can indicate a misaligned filter, which could lead to incorrect results)
	E05 - Failed "quality checks" (All of the above tests pass, but any one of the following is true: the pattern-matching algorithms failed to find the filter, the filter is too bright or too dark, or the standard deviation of the image is too high relative to the image's intensity.)

Example of a run report that includes all the possible result reporting:



Meaning of Error or Indeterminate Codes

An ERROR result will be displayed by the Encompass MDx Workstation if any of the following occurred during the performance of the assay:

- The Reference Spot failed
- The assay was aborted due to workstation or consumable failure.
- Insufficient data were collected.

An INDETERMINATE result will be displayed if any of the following occurred during the performance of the assay:

- A target-specific PCR and Process Control failed.
- Errors in the intensity or quality of one or more of the target spots on the integrated DNA array.

If an ERROR or INDETERMINATE test result is reported, repeat the test by obtaining the original specimen transport tube and repeat the test only for the target reported as ERROR or INDETERMINATE using a new CARD cartridge and reagents. If an ERROR Code persists, please contact Rheonix. If an INDETERMINATE code persists, obtain a fresh specimen and retest.

Limitations

- The Rheonix STI TriPlex Assay may only be performed using the Rheonix Encompass MDx Workstation using clinical specimens that have been collected using the Rheonix Urine Specimen Collection Kit. Specimens collected using any other manufacture's collection devices cannot be analyzed with this kit.
- Performance of the assay has not been established in female urine.
- Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions provided in this package insert may cause erroneous results.
- Reliable results are dependent on adequate specimen collection. Because the collection and transport system does not allow for microscopic assessment of specimen adequacy, training of clinicians in proper specimen collection techniques is necessary. Please refer to the package insert of the Rheonix Urine Specimen Collection Kit for more information.
- Careful compliance with the instructions in this package insert and to the Rheonix Urine Specimen Collection Kit package insert are necessary to avoid erroneous results.
- The Rheonix STI TriPlex Assay has not been validated for use with specimens collected by patients at home
- Talcum Powder may interfere with the Rheonix STI TriPlex assay when at concentrations higher than 0.6% w/v.
- Though rare, mutations within the highly-conserved regions covered by the primers and/or probes of the Rheonix STI TriPlex Assay may result in failure to detect the presence of the organism(s).
- The assay has not been evaluated with patients who are currently being treated with antimicrobial agents active against CT, NG, TV.
- The assay should not be used to evaluate the success or failure of a therapeutic intervention.
- The assay has not been evaluated for patients younger than 14 years of age.
- The assay must be performed on first-catch urine specimens (defined as the first 20-30 mL of the urine stream).
- Use of the assay is not approved for the evaluation of suspected sexual abuse contact tracings as well as for other medico-legal indications.
- As with any diagnostic test, results from the assay should be interpreted in conjunction with other clinical and laboratory findings.

Expected Values

The prevalence of infection with CT, NG, and/or TV in patient populations depends on factors such as age, gender, the presence or absence of symptoms, clinic, and the sensitivity of the test used to detect infections. During the clinical evaluation of the Rheonix STI TriPlex™ Assay, the positivity rate for detection of CT, NG, and/or TV using the Rheonix STI TriPlex™ Assay, by clinical study site and overall, is shown in the table below:

Collection Site	Positivity Rate as Determined by Rheonix STI TriPlex™ Assay by Clinical Site		
	CT	NG	TV
1	19.5%	17.7%	7.1%
2	4.6%	7.2%	0.0%
3	9.7%	4.7%	1.7%
4	13.2%	5.0%	1.3%
5	10.9%	2.0%	2.0%
6	8.7%	10.6%	5.9%
7	1.9%	4.8%	0.0%
8	23.1%	23.1%	0.0%
All Sites	9.8%	7.0%	2.2%

Performance Characteristics

During the clinical study to evaluate the performance of the Rheonix STI TriPlex Assay, specimens from a total of 1627 male subjects (aged 14 years and older) were collected at 8 geographically distinct sites in US. The study enrolled both symptomatic and asymptomatic subjects. One first-catch urine specimen was collected from each subject in a healthcare setting. The collected specimen from each subject was subsequently aliquoted and transferred into a total of four different manufacturers' transport tubes (one from Rheonix Urine Specimen Collection kit and three others for reference methods). All processed urine specimens were shipped via overnight courier to a central laboratory for Patient Infection Status (PIS) testing and then distributed to one of three separate testing laboratories for test by Rheonix STI TriPlex assay.

Out of the total of 1627 subjects, 14 were excluded from test for reasons including ineligibility issues (N = 1), Patient Withdrawal (N=3), sample mishandling/shipping issues (N=10). Of the remaining 1613 evaluable subjects, 1606 were evaluated for CT and NG (7 subjects were excluded from performance analysis due to testing noncompliance or unevaluable PIS testing results), while 1586 subjects were evaluated for TV (27 subjects were excluded from performance analysis due to testing noncompliance or unevaluable PIS testing results). Of the 1586 subjects evaluated for TV, results from one subject were not included in the calculation of Sensitivity and Specificity for TV because of invalid test results in Rheonix STI TriPlex Assay. Therefore, results from a total of 1585 subjects were used to calculate the Sensitivity and Specificity of the Rheonix STI TriPlex Assay for the TV target while results from a total of 1606 subjects were used to calculate the Sensitivity and Specificity for the CT and NG targets.

The following is the prevalence of each pathogen at each clinical study site and all sites combined based on the comparator results.

Collection Site	Positivity Rate based on PIS		
	CT	NG	TV
1	20.4%	18.6%	7.1%
2	4.6%	7.2%	0.0%
3	9.7%	4.7%	1.7%
4	13.5%	5.0%	1.6%
5	12.2%	2.0%	2.0%
6	9.1%	10.6%	5.1%
7	1.9%	4.8%	0.0%
8	23.1%	23.1%	0.0%
All Sites	10.1%	7.1%	2.2%

Male subjects were classified as Infected or Not Infected according to the following algorithm:

Male urine specimens were analyzed by up to three different FDA-cleared Nucleic Acid Amplification Tests (NAATs) for the presence of CT, NG and TV. Only two tests were utilized if their results were concordant. When discordant results were obtained, a third "tie-breaker" NAAT was performed. A Positive Patient Infection Status (PIS) was established when at least two comparator NAATs yielded positive results. A Negative PIS was established when at least two comparator NAATs yielded negative results. All other assay result combinations were reported as indeterminate and were excluded from analysis.

Based upon comparison against the PIS of each enrollee, the following performance estimates were calculated for each of the analytes:

Gender	Specimen	Symptom	CT						
			N	TP	FP	TN	FN	%Sens (95% CI)	%Spec (95% CI)
Male	Urine	A	1202	96	0	1104	2	98.0% (92.9% - 99.4%)	100.0% (99.7% - 100.0%)
		S	404	62	0	339	3	95.4% (87.3% - 98.4%)	100.0% (98.9% - 100.0%)
		ALL	1606	158	0	1443	5	96.9% (93.0% - 98.7%	100.0% (99.7% - 100.0%)

Gender	Specimen	Symptom	NG						
			N	TP	FP	TN	FN	%Sens (95% CI)	%Spec (95% CI)
Male	Urine	A	1201	14	0	1187	0	100.0% (78.5% - 100.0%)	100.0% (99.7% - 100.0%)
		S	405	99	0	305	1	99% (94.6% - 99.8%)	100.0% (98.8% - 100.0%)

		ALL	1606	113	0	1492	1	99.1% (95.2% - 99.8%)	100.0% (99.7% - 100.0%)

Gender	Specimen	Symptom	TV						
			N	TP	FP	TN	FN	%Sens (95% CI)	%Spec (95% CI)
Male	Urine	A	1187	22	1	1163	1	95.7% (79.0% - 99.2%)	99.9% (99.5% - 100.0%)
		S	398	12	0	386	0	100% (75.8% - 100.0%)	100% (99.0% - 100.0%
		ALL	1585*	34	1	1549	1	97.1% (85.5% - 99.5%)	99.9% (99.6% - 100.0%)

*One of the tested 1586 subjects was excluded from the above performance analysis due to the lack of valid result for Rheonix STI TriPlex assay

Comparison of Rheonix Results for *Chlamydia trachomatis* in males against all possible PIS Outcomes

PIS	PIS Tests			Rheonix Result	Symptoms		
	Test 1*	Test 2*	Test 3*		[a]CT_SX	[b]CT_ASX	[c]CT_Total
Not Infected	-	-	NA	-	333	1098	1431
	-	+	-	-	1	5	6
	+	-	-	-	2	1	3
	Invalid	-	-	-	3	0	3
	-	-	NA	+	0	0	0
	-	+	-	+	0	0	0
	+	-	-	+	0	0	0
	-	-	NA	Invalid	0	0	0
	-	+	-	Invalid	0	0	0
	+	-	-	Invalid	0	0	0
Total Not Infected					339	1104	1443
Infected	+	+	N/A	+	60	95	155
	+	-	+	+	2	0	2
	-	+	+	+	0	0	0
	+	Invalid	+	+	0	1	1
	+	+	N/A	-	2	2	4
	+	-	+	-	0	0	0
	-	+	+	-	1	0	1
	+	+	N/A	Invalid	0	0	0
	+	-	+	Invalid	0	0	0
	-	+	+	Invalid	0	0	0
Total Infected					65	98	163

[a]*CT_SX (Symptomatic Males)*
[b]*CT_ASX (Asymptomatic Males)*
[c]*CT_Total (Total Male enrollees)*

Comparison of Rheonix Results for *Neisseria gonorrhoeae* in males against all possible PIS Outcomes

PIS	PIS Tests			Rheonix Result	Symptoms		
	Test 1*	Test 2*	Test 3*		[a]NG_SX	[b]NG_ASX	[c]NG_Total
Not Infected	-	-	NA	-	305	1185	1490
	-	+	-	-	0	1	1
	+	-	-	-	0	1	1
	Invalid	-	-	-	0	0	0
	-	-	NA	+	0	0	0
	-	+	-	+	0	0	0
	+	-	-	+	0	0	0
	-	-	NA	Invalid	0	0	0
	-	+	-	Invalid	0	0	0
	+	-	-	Invalid	0	0	0
Total Not Infected					305	1187	1492
Infected	+	+	N/A	+	99	14	113
	+	-	+	+	0	0	0
	-	+	+	+	0	0	0
	+	Invalid	+	+	0	0	0
	+	+	N/A	-	1	0	1
	+	-	+	-	0	0	0
	-	+	+	-	0	0	0
	+	+	N/A	Invalid	0	0	0
	+	-	+	Invalid	0	0	0
	-	+	+	Invalid	0	0	0
Total Infected					100	14	114

[a]*NG_SX (Symptomatic Males)*
[b]*NG_ASX (Asymptomatic Males)*
[c]*NG_Total (Total Male enrollees)*

Comparison of Rheonix Results for *Trichomonas vaginalis* in males against all possible PIS Outcomes

PIS	PIS Tests			Rheonix Result	Symptoms		
	Test 1*	Test 2*	Test 3*		[a]TV_SX	[b]TV_ASX	[c]TV_Total
Not Infected	-	-	NA	-	382	1161	1543
	-	+	-	-	1	2	3
	+	-	-	-	1	0	1
	Invalid	-	-	-	2	0	2
	-	-	NA	+	0	0	0
	-	+	-	+	0	1	1
	+	-	-	+	0	0	0
	-	-	NA	Invalid	0	1	1
	-	+	-	Invalid	0	0	0
	+	-	-	Invalid	0	0	0
Total Not Infected					386	1165	1551
Infected	+	+	N/A	+	12	22	34
	+	-	+	+	0	0	0
	-	+	+	+	0	0	0
	+	Invalid	+	+	0	0	0
	+	+	N/A	-	0	1	1
	+	-	+	-	0	0	0
	-	+	+	-	0	0	0
	+	+	N/A	Invalid	0	0	0
	+	-	+	Invalid	0	0	0
	-	+	+	Invalid	0	0	0
Total Infected					12	23	35

[a]*TV_SX (Symptomatic Males)*
[b]*TV_ASX (Asymptomatic Males)*
[c]*TV_Total (Total Male enrollees)*

Hypothetical Positive Predictive Values/Negative Predictive Values

The hypothetical Positive Predictive Values and Negative Predictive Values were calculated and are based upon the Clinical Sensitivity and Clinical Specificity observed in the clinical studies of the Rheonix STI TriPlex Assay and hypothetical prevalence rates for the three targets of interest.

Specimen Type	Hypothetical Prevalence	Chlamydia trachomatis				Neisseria gonorrhoeae				Trichomonas vaginalis			
		% Sen	% Spec	% PPV	% NPV	% Sen	% Spec	% PPV	% NPV	% Sen	% Spec	% PPV	% NPV
Male Urine	1%	96.9%	100%	100.0%	100.0%	99.1	100.0	100.0%	100.0%	97.1	99.9	90.8%	100.0%
	2%			100.0%	99.9%			100.0%	100.0%			95.2%	99.9%
	5%			100.0%	99.8%			100.0%	100.0%			98.1%	99.9%
	10%			100.0%	99.7%			100.0%	99.9%			99.1%	99.7%
	15%			100.0%	99.5%			100.0%	99.8%			99.4%	99.5%
	20%			100.0%	99.2%			100.0%	99.8%			99.6%	99.3%
	25%			100.0%	99.0%			100.0%	99.7%			99.7%	99.0%

Repeat and Unresolved Rates

The Rheonix Encompass MDx workstation reports results as Positive, Negative or Indeterminate (IND) for each of the three target microorganisms. In addition, the workstation also reports a variety of error codes (ERR) that would require testing. On a target-by-target basis, the combined IND and ERR code rates are reported. The values reported as "Initial" represent samples that displayed either an IND or ERR code that required a reanalysis. The "Unresolved" values represent those samples that, upon repeat, did not yield valid results. As noted, of all male subjects, only one urine specimen yielded an unresolved final result, also representing 0.06% of the male population evaluated. All other initially failed runs yielded valid, interpretable results upon repeat testing.

Rates of Initial and Final Unresolved Results by Rheonix STI TriPlex assay

Subject	*Chlamydia trachomatis*			*Neisseria gonorrhoeae*			*Trichomonas vaginalis*		
	Total N	Initial	Unresolved	Total N	Initial	Unresolved	Total N	Initial	Unresolved
Male	**1606**	20 (1.3%)	0 (0.0%)	**1606**	16 (1.0%)	0 (0.0%)	**1586**	24 (1.5%)	1 (0.1%)
		95% CI			**95% CI**			**95% CI**	
		(0.8% - 1.9%)	(0.0% - 0.2%)		(0.6% - 1.6%)	(0.0% - 0.2%)		(1.0% - 2.2%)	(0.0% - 0.04%)

ANALYTICAL PERFORMANCE

Analytical Sensitivity

The analytical sensitivity (i.e., Limit of Detection, LoD) for the Rheonix STI TriPlex assay in urine specimens was determined as follows: Two representative microbial strains/serovars for each of the three targets (i.e., CT, NG and TV) were prepared at multiple concentrations in negative urine matrix. Each suspension was quantified prior to analysis. The LoD was first estimated by probit analysis by testing six concentrations of each organism in 26 replicates by three different lots of assay kit. The calculated LoD for each strain was verified by testing 44 replicates at the estimated concentration and demonstrating that at least 95% of the replicates (42 out of 44) were positive. If the criteria of at least 42 out of 44 positive replicates were not met, the results were added to the data set and re-analyzed to obtain a new estimated LoD value. Additional replicates were tested at the revised estimate and the process was repeated until at least 95% of all replicates gave a positive test result.

Based on this analysis, the Analytical Sensitivity of the Rheonix STI TriPlex assay in urine specimens for each of the three targets was established:

Limit of Detection in Urine Matrix		
Target	**Serovar or Strain**	**Limit of Detection**
Chlamydia trachomatis	Serovar D	19 IFU/ml
	Serovar H	26 IFU/ml
Neisseria gonorrhoeae	ATCC 49226	180 CFU/ml
	ATCC 19424	110 CFU/ml
Trichomonas vaginalis	ATCC 30236*	4 Trophozoites/ml
	ATCC 50143**	5 Trophozoites/ml

** Metronidazole (MTZ) sensitive*
***MTZ resistant*

Inclusivity

Once the LoD was established, an additional 13 serovars of CT, 30 strains of NG and 6 strains of TV were tested in 20 replicates at the most challenging LoDs noted above for each target in pooled urine matrix. If the initial concentration tested yielded lower than 95% positive results for a particular strain, then additional replicates were tested at higher target concentration.

For CT: of all 13 additional strains tested, 11 were detected at 3xLoD or lower target concentrations, and strains L1 and Ba were detected at higher concentrations with ≥95% positivity, as shown in the table below.

For NG: all 30 strains of NG were detected at 95% positive rate or higher at 110 CFU/mL in urine matrix.

For TV: all six strains of TV were detected at 95% positive rate or higher at 4 trophozoites/mL in urine matrix.

The results of the inclusivity study are shown below.

Results for Additional Strains of CT

CT Serovar	IFU/mL	x LoD	%Pos
A	11	0.6	100
B	11	0.6	100
C	33	1.7	100
E, nvCT	22	1.2	100
F	11	0.6	100
I	22	1.2	100
J	55	2.9	100
K	33	1.7	95
G	33	1.7	100
L1	154	8.1	100
L2	11	0.6	100
L3	55	2.9	100
Ba	110	5.8	95

Results for Additional Strains of NG

NG Strain	CFU/ml	x LoD	%Pos	NG Strain	CFU/ml	x LoD	%Pos
Z423	110	1	100	**Z438**	110	1	100
Z424	110	1	100	**Z439**	110	1	100
Z425	110	1	100	**Z440**	110	1	100
Z426	110	1	100	**Z441**	110	1	100
Z427	110	1	100	**Z442**	110	1	100
Z428	110	1	95	**Z443**	110	1	100
Z429	110	1	100	**Z444**	110	1	100
Z430	110	1	100	**Z445**	110	1	100
Z431	110	1	100	**Z446**	110	1	100
Z432	110	1	100	**Z448**	110	1	100
Z433	110	1	100	**Z449**	110	1	100
Z434	110	1	100	**Z450**	110	1	100
Z435	110	1	100	**Z451**	110	1	100
Z436	110	1	100	**Z452**	110	1	100
Z437	110	1	100	**Z466**	110	1	100

Results for Additional Strains of TV

TV Strain	Trophozoites/ml	x LoD	%Pos
Z070	4	1	100
CDC252*	4	1	100
Z158	4	1	100
Z159	4	1	100
ATCC 30238	4	1	100
ATCC 30001	4	1	100

*MTZ resistant

Analytical Specificity/Cross Reactivity

A total of 156 non-target microorganisms that could potentially be found in urogenital samples being analyzed for the presence of CT, NG and TV were evaluated in triplicate in pooled urine samples that were previously confirmed to not contain CT, NG or TV when tested with the Rheonix STI TriPlex Assay. Each bacterial non-target organism was tested at 10^6 CFU/ml, while each viral non-target organism was tested at 10^5 PFU/ml.

All microbes tested, with the exception of Herpes Simplex Virus, type 1 (HSV1) and *Neisseria meningitides serogroup D,* returned negative results for all three replicates. When initially tested in triplicate, the HSV1 sample (tested at 10^5 PFU/ml) returned one positive result for TV while the *N. meningitides* (tested at 10^6 CFU/ml) returned one positive result for NG. Upon retest in triplicate, however, all three replicates for each of these two potentially cross-reactive specimens were negative for all three target microbes.

Potentially Cross-reactive microorganisms tested

Microorganisms Tested	
Achromobacter xerosis	*Chlamydophila (Chlamydia) psittaci*
Acinetobacter calcoaceticus	*Chlamydophila (Chlamydia) psittaci*
Acinetobacter lwoffii	*Chromobacterium violaceum*
Actinomyces israelii	*Citrobacter freundii*
Actinomyces pyogenes (Trueperella pyogenes)	*Clostridium difficile*
Aerococcus viridans	*Clostridium perfringens*
Aeromonas hydrophila	*Corynebacterium genitalium*
Alcaligenes faecalis	*Corynebacterium xerosis*
Atopobium vaginae	*Cryptococcus neoformans*
Bacillus subtilis	*Cytomegalovirus (CMV)*
Bacteroides fragilis	*Deinococcus radiodurans*
Bergeriella (Neisseria) denitrificans	*Derxia gummosa*
Bifidobacterium adolescentis	*Eikenella corrodens*
Bifidobacterium breve	*Elizabethkingia meningoseptica (Flavobacterium melingosepticum)*
Blautia producta (Peptostreptococcus productus)	*Enterobacter aerogenes*
Brevibacterium linens	*Enterobacter cloacae*
Campylobacter jejuni	*Enterococcus avium*

Microorganisms Tested	
Campylobacter ureolyticus (Bacteroides ureolyticus)	*Enterococcus faecalis*
Candida albicans	*Enterococcus faecium*
Candida glabrata	*Erysipelothrix rhusiopathiae*
Candida parapsilosis	*Escherichia coli*
Candida tropicalis	*Fusobacterium nucleatum*
Chlamydophila pneumoniae	*Gardnerella vaginalis*
Gemella haemolysans	*Neisseria cinerea*
Haemophilus ducreyi	*Neisseria cinerea*
Haemophilus influenzae	*Neisseria elongate*
Herpes Simplex Virus , type I (HSV1)	*Neisseria elongate*
Herpes Simplex Virus , type II (HSV2)	*Neisseria elongate*
HIV type 1	*Neisseria flavescens*
HPV 16	*Neisseria flavescens*
Weissella paramesenteroides (Leuconostoc paramensenteroides)	*Neisseria lactamica*
Human Papilloma Virus 6	*Neisseria lactamica*
Kingella denitrificans	*Neisseria lactamica*
Kingella kingae	*Neisseria lactamica*
Klebsielia oxytoca	*Neisseria lactamica*
Klebsiella pneumoniae	*Neisseria lactamica*
Lactobacillus acidophilus	*Neisseria lactamica*
Lactobacillus brevis	*Neisseria lactamica*
Lactobacillus crispatus	*Neisseria lactamica*
Lactobacillus jensenii	*Neisseria meningitidis serogroup A*
Lactobacillus debrueckii (lactis)	*Neisseria meningitidis serogroup B*
Lactobacillus vaginalis	*Neisseria meningitidis serogroup C*
Legionella pneumophila	*Neisseria meningitidis serogroup C*
Legionella pneumophila	*Neisseria meningitidis serogroup C*
Listeria monocytogenes	*Neisseria meningitidis serogroup C*
Micrococcus luteus	*Neisseria meningitidis serogroup D*
Mobiluncus curtisii	*Rhodospirillum rubrum*
Moraxella (Branhamella) catarrhalis	*Saccharomyces cerevisiae*
Moraxella lacunata	*Yersinia enterocolitica*
Moraxella osloensis	*Neisseria meningitidis serogroup W135*
Morganella morganii	*Neisseria meningitidis serogroup Y*
Mycobacterium smegmatis	*Neisseria mucosa*
Mycoplasma genitalium	*Neisseria mucosa*
Mycoplasma hominis	*Neisseria mucosa*
Neisseria cinerea	*Neisseria polysaccharea*
Neisseria cinerea	*Neisseria sicca*
Neisseria sicca	*Pseudomonas aeruginosa*
Neisseria sicca	*Pseudomonas fluorescens*
Neisseria subflava biovar flava	*Pseudomonas putida*
Neisseria subflava biovar flava	*Rahnella aquatilis*
Neisseria subflava biovar perflava	*Rhizobium (Agrobacterium) radiobacter*
Neisseria subflava biovar perflava	*Salmonella enterica Minnesota*
Neisseria subflava biovar perflava	*Salmonella typhimurium*
Neisseria subflava biovar perflava	*Serratia marcescens*
Neisseria subflava biovar perflava	*Staphylococcus aureus*

Microorganisms Tested	
Neisseria subflava biovar perflava	*Staphylococcus epidermidis*
Neisseria subflava biovar subflava	*Staphylococcus saprophyticus*
Pantoea agglomerans (Erwinia herbicola)	*Streptococcus agalactiae*
Paracoccus denitrificans	*Streptococcus bovis*
Pentatrichomonas hominis	*Streptococcus mitis*
Peptostreptococcus anaerobius	*Streptococcus mutans*
Peptostreptococcus magnus (Finegoldia magna)	*Streptococcus pneumoniae*
Plesiomonas shigelloides	*Streptococcus pyogenes*
Prevotella bivia	*Streptococcus salivarius*
Propionibacterium acnes	*Streptococcus sanguinis*
Proteus mirabilis	*Trichomonas tenax*
Proteus vulgaris	*Ureaplasma urealyticum*
Providencia stuartii	*Vibrio parahaemolyticus*

Interfering Substances

A total of 26 potentially interfering substances that could be present in urogenital tract specimens were evaluated at concentrations selected to be medically relevant. The testing was performed by analyzing pooled male urine that contained a mixture of the three targets spiked at 1.5 x their respective LoDs. Since a small percentage of samples analyzed at 1.5 x LoD would be expected to return negative results, even in the absence of any interfering substances, if potential interference was observed at 1.5 x LoD, the potential interfering substance was retested using the matrix spiked with 3 x their respective LoDs. None of the substances tested yielded interference at the concentrations noted in the following tables.

Substances Tested in Urine Matrix	
Interfering substance	**Concentration**
Whole Blood	2% (v/v)
Semen	5% (v/v)
Hormones	0.48 ng/mL 17-α-Ethinylestradiol
AntiProtozoal (Metronidazole)	48 µg/mL
Glucose	0.48 mg/mL
Acetylsalicylic Acid	260.8 µg/mL
Azithromycin	4.8 µg/mL
Phenazopyridine Hydrochloride	80 µg/mL
Norithindrone	8 ng/mL
4-Acetaminophenol	80 µg/mL
Naproxen	200 µg/mL
Ibuprofen	200 µg/mL
Amoxicillin Trihydrate	30.08 µg/mL
Tetracycline Hydrochloride	6 µg/mL
Ceftriaxone	324.4 µg/mL
Sulfamethoxazole	160 µg/mL
Trimethoprim	16 µg/mL L
Erythromycin	24 µg/mL
Human Serum Albumin	0.4 mg/mL

Substances Tested in Urine Matrix	
Interfering substance	**Concentration**
Leukocytes	10^6 cells/mL
Feminine Deodorant Spray	0.68% v/v
Talcum Powder[a]	0.6% w/v
Bilirubin	0.08 mg/mL
Biotin	3500 ng/ml
Urine (high pH)	pH 9
Urine (low pH 4)	pH 4

[a]*May interfere with the Rheonix STI TriPlex assay when at concentrations higher than shown.*

Mixed Infection/Competitive Interference

The mixed infection/competitive interference study was designed to evaluate the ability of the Rheonix STI TriPlex Assay to detect low positive results in the presence of the other targets at high concentrations in urine matrix. Two (2) organisms (*Neisseria gonorrhoeae* and *Trichomonas vaginalis*) were individually prepared at 1.5X their respective LoD and *Chlamydia trachomatis* was prepared at 1.7X its respective LoD to serve as a low target in the Rheonix STI Collection Buffer. When added as a high target to the mixture, CT was added at 10^6 IFU/ml, NG was added at 10^6 CFU/ml and TV was added at 10^5 trophozoites/ml. No interference was observed when either CT, NG or TV were tested in the presence of exceedingly high concentrations of the other two targets.

Precision and Reproducibility

Precision

The objective of this study was to establish the intra-laboratory precision of the Rheonix STI TriPlex Assay as performed on the Rheonix Encompass MDx™ Workstation. The study was divided into two arms. Study A investigated potential variability due to days, runs, and operators, while Study B investigated variability due to potential lot-to-lot variation in Rheonix STI TriPlex Assay kits (i.e., CARD cartridges and reagents). The blinded Precision Test Panel (PTP) contained male urine specimen matrix individually spiked with CT, NG or TV at the concentrations specified below. Within-laboratory precision of the Rheonix STI TriPlex assay was evaluated at one (1) site, with each member of the panel evaluated in duplicate. Three separate operators performed the tests using one Encompass MDx™ instrument. Each operator performed two different runs/day for 3 non-consecutive days over a 12-day period.

For the lot-to-lot variability, each member of the PTP panel was evaluated in duplicate, by one operator, using one Encompass MDx™ instrument, performing two different runs/day for 3 non-consecutive days over a 12-day period. A total of three different lots of Rheonix CT/NG/TV CARDs and reagents were tested.

- Moderate Positive (MP): 5 x LoD
- Low Positive (LP): 2 x LoD
- High Negative (HN): 0.25 x LoD
- True Negative (TN): No targets present

The Positive and Negative agreements when detecting each of the three targets in male urine are reported below.

Overall Precision Study Results Using One Lot of the Rheonix STI TriPlex Assay Kit

Panel Member	Percent (%) Observed versus Expected		
	C. trachomatis	*N. gonorrhoeae*	*T. vaginalis*
	Urine	Urine	Urine
[a]TN	97.2% (35/36) 85.8% - 99.5%	100% (36/36) 90.4% - 100%	100% (36/36) 90.4% - 100%
[b]HN	33.3% (12/36) 20.2% - 49.7%	85.3% (29/34) 69.9% - 93.6%	72.2% (26/36) 56.0% - 84.2%
LP	91.7% (33/36) 78.2% - 97.1%	100% (36/36) 90.4% - 100%	100% (36/36) 90.4% - 100%
MP	97.2% (35/36) 85.8% - 99.5%	97.2% (35/36) 85.8% - 99.5%	100% (36/36) 90.4% - 100%

[a]*For the True Negative (TN) category, the reported agreement indicates the percent of negative results.*
[b]*For the High Negative (HN) category, the reported agreement indicates the percent of positive results.*

The results for Lot-to-lot variability indicated that the vast majority of variability was due to replicate-to-replicate variation within the run, while very little, if any variation was caused by differences in the three lots employed.

Lot-to-Lot Reproducibility

Target	Sample Type	Panel Member ID	Correct	Total	% Correct	95% CI
CT	Urine	TN*	36	36	100	90.4% - 100%
		HN**	14	36	38.9	24.8% - 55.1%
		LP	36	36	100	90.4% - 100%
		MP	35	36	97.2	85.8% - 99.5%
NG		TN*	36	36	100	90.4% - 100%
		HN**	7	35	20.0	10.0% - 35.9%
		LP	36	36	100	90.4% - 100%
		MP	36	36	100	90.4% - 100%
TV		TN*	36	36	100	90.4% - 100%
		HN**	10	36	27.8	15.9% - 44.0%
		LP	36	36	100	90.4% - 100%
		MP	36	36	100	90.4% - 100%

**TN samples do not contain any target analytes. Therefore "% Correct" refers to the percent of negative test results.*

***HN samples, the "% Correct" refers to the percent of positive results.*

Reproducibility

For Site-to-Site reproducibility, three sites (two external, one internal) were provided with the same blinded panels described for the Precision Study. Two operators at each site performed the testing by analyzing each

panel member in duplicate twice each day over a period of five non-consecutive days.

Percent Overall Agreement

Percent overall agreement (across sites, operators, days, runs and within run) is presented for each target in urine matrix.

Panel Member	Percent (%) Observed versus Expected		
	C. trachomatis	*N. gonorrhoeae*	*T. vaginalis*
	Urine	Urine	Urine
[a]TN	100% (120/120) 96.9% - 100%	100% (120/120) 96.9% - 100%	100% (120/120) 96.9% - 100%
[b]HN	44.1% (52/118) 35.4% - 53.1%	79.2% (95/120) 71.1% - 85.5%	78.8% (93/118) 70.6% - 85.2%
LP	100% (120/120) 96.9% - 100%	100% (120/120) 96.9% - 100%	100% (120/120) 96.9% - 100%
MP	100% (120/120) 96.9% - 100%	100% (120/120) 96.9% - 100%	100% (120/120) 96.9% - 100%

[a]*For the True Negative (TN) category, the reported agreement indicates the percent of negative results.*
[b]*For the High Negative (HN) category, the reported agreement indicates the percent of positive results.*

Percent Agreement by Site

The percent agreement by site for each target in urine matrix is provided.

Percent Agreement with Expected Result by Site for Qualitative CT Results in Urine

Sample ID	Site	Sample Size	Number Positive	Percent Positive Tests	95% CI
TN	1	40	0	0.0%	(0.0%, 8.8%)
	2	40	0	0.0%	(0.0%, 8.8%)
	3	40	0	0.0%	(0.0%, 8.8%)
HN	1	40	18	45.0%	(30.7%, 60.17%)
	2	40	20	50.0%	(35.2%, 64.8%)
	3	38	14	36.8%	(23.4%, 52.7%)
LP	1	40	40	100.0%	(91.2%, 100.0%)
	2	40	40	100.0%	(91.2%, 100.0%)
	3	40	40	100.0%	(91.2%, 100.0%)
MP	1	40	40	100.0%	(91.2%, 100.0%)
	2	40	40	100.0%	(91.2%, 100.0%)
	3	40	40	100.0%	(91.2%, 100.0%)

Percent Agreement with Expected Result by Site for Qualitative NG Results in Urine

Sample ID	Site	Sample Size	Number Positive	Percent Positive Tests	95% CI
TN	1	40	0	0.0%	(0.0%, 8.8%)
	2	40	0	0.0%	(0.0%, 8.8%)
	3	40	0	0.0%	(0.0%, 9.0%)
HN	1	40	34	85.0%	(70.9%, 92.9%)
	2	40	31	77.5%	(62.5%, 87.7%)
	3	40	30	75.0%	(59.8%, 85.8%)
LP	1	40	40	100.0%	(91.2%, 100.0%)
	2	40	40	100.0%	(91.2%, 100.0%)
	3	40	40	100.0%	(91.2%, 100.0%)
MP	1	40	40	100.0%	(91.2%, 100.0%)
	2	40	40	100.0%	(91.2%, 100.0%)
	3	40	40	100.0%	(91.2%, 100.0%)

Percent Agreement with Expected Result by Site for Qualitative TV Results in Urine

Sample ID	Site	Sample Size	Number Positive	Percent Positive Tests	95% CI
TN	1	40	0	0.0%	(0.0%, 8.8%)
	2	40	0	0.0%	(0.0%, 8.8%)
	3	40	0	0.0%	(0.0%, 9.0%)
HN	1	40	32	80.0%	(65.2%, 89.5%)
	2	40	34	85.0%	(70.9%, 92.9%)
	3	40	27	67.5%	(52.0%, 79.9%)
LP	1	40	40	100.0%	(91.2%, 100.0%)
	2	40	40	100.0%	(91.2%, 100.0%)
	3	40	40	100.0%	(91.2%, 100.0%)
MP	1	40	40	100.0%	(91.2%, 100.0%)
	2	40	40	100.0%	(91.2%, 100.0%)
	3	40	40	100.0%	(91.2%, 100.0%)

Carry Over/Cross-contamination

A study was conducted to investigate within-run carryover and between-run carryover while processing samples with high microbial loads of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* in the Rheonix STI TriPlex Assay. Two different composite samples were tested. One panel member consisted of all three targets at high concentration (*Chlamydia trachomatis,* serovar D at 10^6 IFU/ml, *Neisseria gonorrhoeae,* ATCC strain 49226, at 10^6 CFU/ml and *Trichomonas vaginalis,* ATCC strain 30236 at 10^5 trophozoites/mL) and a second panel member consisted of only the matrix without any spiked targets. The high positive samples were run in a CARD cartridge lane immediately adjacent to lane that contained the negative sample. A total of 144 alternating samples were run across nine days and six runs on a single Rheonix Encompass MDx Workstation. In all cases, the high positive sample yielded positive results for all targets in each run while the

negative sample yielded negative results for all targets in each run. Therefore, no carry over or cross contamination was observed.

Labeling Symbology

Symbol	Title of symbol	Explanatory Text	Standard Reference
	Manufacturer	Indicates the medical device manufacturer	ISO 15223-1:2016 Reference no. 5.1.1
	Date of Manufacture	Indicates the date when the medical device was manufactured	ISO 15223-1:2016 Reference no. 5.1.3 ISO 7000:2014 Reference no. 2497
	Consult instructions for use	Indicates the need for the user to consult the instructions for use	ISO 15223-1:2016 Reference no. 5.4.3 ISO 7000:2014 Reference no.1641
	Temperature limits	Indicates the temperature limits to which the medical device can be safely exposed.	ISO 15223-1: 2016 Reference number 5.3.7 ISO 7000:2014 Reference no. 0632
	Do not use if damaged	Indicates a medical device that should not be used if the package has been damaged or opened	ISO 15223-1: 2016 reference number 5.2.8 ISO 7000:2014 Reference no. 2606
	Single use only; do not reuse	Indicates a medical device that is intended for one use, or for use on a single patient during a single procedure	ISO 15223-1:2016 Reference no. 5.4.2 ISO 7000:2014 Reference no. 1051
	Caution, consult accompanying documents	Indicates the need for the user to consult the instructions for use for important cautionary information	ISO 15223-1:2016 Reference no. 5.4.4 ISO 7000:2014 Reference no. 0434
	Contains sufficient contents for <n> tests	Indicates the total number of IVD tests that can be performed with the IVD	ISO 15223-1:2016 Reference no. 5.5.5 ISO 7000:2014 Reference no. 0518
	*In vitro* diagnostic medical device	Indicates a control material that is intended to verify the performance characteristics of another medical device	ISO 15223-1:2016 Reference no. 5.5.2 ISO 7000:2014 Reference no. 2494

Symbol	Title of symbol	Explanatory Text	Standard Reference
	Prescription use only	Caution: Federal law (USA) restricts this device to sale by or on the order of a licensed healthcare practitioner	21 CFR 801.109
	Catalog Number	Indicates the manufacturer's catalogue number to identify the medical device	ISO 15223-1:2016 Reference no. 5.1.6 ISO 7000:2014 Reference no. 2493
	Lot Number	Indicates the manufacturer's batch code to identify the batch or lot	ISO 15223-1:2016 Reference no. 5.1.5 ISO 7000:2014 Reference no. 2492
	Environmental or aquatic toxicity	Indicates a potential of environmental or aquatic toxicity	US: 29 CFR 1910:1200 (HCS) EU: Regulation 1272/2008/EC GHS 09
	Use by	Indicates the date after which the medical device is not to be used	ISO 15223-1:2016 Reference no. 5.1.4 ISO 7000:2014 Reference no. 2607
	Skin Irritation, category 2 Eye Irritation, category 2	Indicates a potential for health risk to the user of the medical device	US: 29 CFR 1910:1200 (HCS) EU: Regulation 1272/2008/EC GHS 07

Intellectual Property

The STI TriPlex™ Assay is covered by the following patents (US patents referenced unless otherwise noted):

Workstation: US 7,976,795; US 8,101,428; US 8,383,039; US 8,609,039; US 9,151,701; CN 102906573; JP 6058399; JP 6104327; US 9,096,890; US 8,986,614; US9,102,979; US 9,328,381; US 9,556,478; AU 2011221244

CARD Cartridges: US 7,608,160; US 7,832,429; US 7,837,821; US 7,959,875; US 8,057,629; US 8,293,053; US 8,323,586; US 8,512,502; US 8,535,020; US 8,646,482; US 8,715,446; US 8;715;447; US 8,763,641; AU 2006320916; AU 2007207681; CN 101282789; EP 1,706,467 (CH, DE, FR, GB, IT & SE); IN 255971; JP 4,516,606; JP 4,939,541; JP 5,250,425; JP 5,323,747; IN 262645; EP 2,520,367 (CH, DE, FR, GB, IT & SE); US 9,638,338; CN 101495236; IN 281148; AU 2007207681; US 8,372,355; US 8,778,280; US 9,134,207; US 9,132,398; CN 101903104; JP 5,523,327; IN 277018;

The PCR and Process Control (PPC) are manufactured by Maine Molecular Quality Controls, Inc.